

Original Research Article

Oral *C.albicans* colonization and Non-*Candida albicans* *Candida* colonization among University students, Yemen

Abstract

Purpose: *Candida albicans* is diploid yeast that in some circumstances may cause oral or oropharyngeal infections. This investigation aimed to study the oral *C.albicans* colonization (OCC) and Non-*Candida albicans* *Candida* colonization (ONCACC) and risk factors of OCC in healthy University students.

Materials and Methods: This cross sectional laboratory study was carried between January 2014 and July 2014. A total of 265 healthy students were included in this study, 131 males and 134 females. Demographic and clinical and risk factor variables were registered in predesigned questionnaire. Standard methods were used for collection oral specimens, culturing and identifying *Candida* species.

Results: The crude rate of OCC was 17.7% and the crude rate of ONCACC was 29.1%. *C.tropicalis* and *C.glabrata* were the most common species isolated after *C.albicans*. Statistically significant association of OCC (< 0.05), was identified between the gender (male) (OR = 3.7), smoking (OR = 14.6), denture wearing (OR = 6.2), dental bridge (OR = 5.4), orthodontics (OR = 2.5), the reduced saliva flow rate (OR = 11.3), previous antibiotics users (OR=2.99), and Qat chewers (OR = 5.2).

Conclusion: Our results are important for the development of strategies to eliminate these indicators of risk and significantly reduce *Candida* species colonization and oral *Candida* infections in young healthy adults and in general in Yemen community. The data also suggests that the prevalence rate of OCC was relatively high and it was affected by presence of prostheses, orthodontics, behaviors, *xerostomia* certain sociodemographic characteristics, which indicate the need for comprehensive, scheduled programs of healthcare educations.

Keywords: Oral *C.albicans* colonization (OCC), Non-*Candida albicans* *Candida* colonization (ONCACC), risk factors, Yemen

Introduction

Candida albicans is an opportunistic fungal pathogen usually found in the human gastrointestinal and female lower genital tracts. It is a only one of its kind parasite capable of colonizing, infecting, and persisting on mucosal surfaces, and motivating mucosal immune responses.[1] Attack of tissues by *Candida* is aided by hyphal development. The transformation of budding yeasts to hyphal growth is endorsed by physical contact with surfaces and is under genetic control. When fungi colonize an epithelial or epidermal surface, they adhere to host cells and generate depressions in the surface of host cells. As yeast-form cells alter to the hyphal form, these hyphae are capable to diffuse into the surface of the tissue layer. The direction of hyphal growth is resolved by the topography of the substratum. Hyphae are guided by ridges in the tissue layer. This behavior is known as thigmotropism. It plays an important role in the direction of hyphal growth and in disease progression .Tissue invasion by *Candida* is made possible by the action of degradative enzymes secreted by the pathogen and by mechanical forces exerted by the hyphae.[2, 3]

C. albicans, is naturally there in the oral cavity in a non-pathogenic state in about one-half of healthy individuals but under favorable situations, has the ability to transform into a pathogenic hyphal form. Conditions that favour this transformation include extremes in

age, broad-spectrum antibiotic therapy, corticosteroids, xerostomia, immune dysfunction (especially of cell-mediated immunity), diabetes mellitus, nutritional deficiencies, or the presence of removable prostheses. [4,5] There are some clinical manifestations of oral candidiasis, the most common being the pseudo membranous, erythematous, angular cheilitis, hyperplastic and mucocutaneous forms.[6,7] Non-*Candida albicans* *Candida* (NCAC) strains, however, are isolated in ever-increasing numbers in medically compromised patients. These strains might cause systemic infections and are frequently resistant to commonly used antifungal agents such as fluconazole.[8,9]. *Candida* species may be able of metabolizing ethanol to carcinogenic acetaldehyde and can thus development oral and upper gastrointestinal tract cancer, consequently, more focus should be placed on diagnosis and treatment of oral *Candida* infections, as well on other *Candida* species than *C. albicans* [8,9,10,11]. This investigation aimed to study the prevalence of *Candida* species and risk factors of oral *Candida albicans* colonization in healthy students of Sana'a University in Sana'a city, Yemen.

Material and methods

Sample collection and processing: The samples were collected aseptically by oral rinse method. The students were asked to rinse the mouth with 10 ml of sterile Phosphate Buffered Saline (PBS, pH 7.2) for 60 seconds and thereafter oral rinse was collected in sterile container. The oral rinse specimen was without delay centrifuged at 3000xg for 10 minute. The supernatant was discarded and sediment was re-suspended in 1 ml of sterile PBS and vortexed for 1 minute. This solution was used for direct microscopic examinations, performed with lactophenol cotton blue and 100 µl of this preparation was inoculated onto Sabouraud's Dextrose Agar (SDA) plate with and without chloroamphenicol (Oxod, UK). The plates were incubated at 37°C for 48 h. The colonies of *Candida* were counted to assess CFU/ml of rinse sample.[12]

Sterile cover-slip, incubated at room temperature for 3 to 5 days in dark to endorse the production of chlamydospores, hyphae, pseudohyphae, and arthroconidia. Biochemical tests were carried out, using API Candida System [13] API system II tests were completed according to the manufacturer's instructions. The API Candida system consists of a single-use disposable plastic strip with 10 wells to perform 12 colorimetric biochemical tests: five sugar assimilation tests (for glucose, galactose, sucrose, trehalose, and raffinose) and seven enzymatic tests (for β -maltosidase, α -amylase, β -xylosidase, β -glucuronidase, urea hydrolysis, *N*-acetyl- β -glucosaminidase, and β -galactosidase). Inoculation of the wells was done by adding a yeast suspension to the dehydrated substrates. The results were read after incubation for 18 to 24 h at 35°C. A four-digit numerical profile was made for each isolate depending upon the reactions it produced. Identifications were made by referring to the list of numerical profiles and a computer program offered by the manufacturer.

The statistical analysis was done using Graph Pad Prism 5. Univariate analyses were performed on all variables of this study using the Fisher's and Chi squared tests (2-sided tests). The results of this analysis were expressed as an odds ratio (OR) with a 95% confidence interval (CI). A *p* value of < 0.05 was considered statistically significant.

Mouth hygiene determined by the frequency of using oral hygiene measures (as mouth washes, using antiplaque and anti-gingivitis tooth paste per day.[14]

Salivary Flow Rate: The students asked to chewing paraffin for 5 minutes, and then saliva collected into a measuring container. Then saliva sample was measured and flow

rate was calculated on an ml/minute basis [15]. It is recommended that the tests are performed at least one hour after the person has eaten something (drinking water is allowed), smoked or taken snuff. It is important that the person is relaxed and calm. If the person has any disease, it should be considered if the disease affects the secretion rate, and if it is a temporary condition or a long-lasting disease. If long-lasting, the reduced secretion rate may be regarded as representative for that person and for that period of time.

All relevant data of the students included in this study were obtained through a pre-designed questionnaire. Also laboratory results, measuring of mouth hygiene and salivary flow rate results were collected in the pre-designed questionnaire.

Result

This analytical laboratory study was conducted on 265 students of Sana'a University during the period of two months from January 2014 to February 2014. Their age was ranged from 20 – 27 years, with mean age \pm SD equal to 22.1 ± 2.1 years for female students, and for male students the mean age \pm SD was 23.4 ± 2.3 years. Females represent 49.4% of total and males represent 50.6% of the total. Most of the students were in age group 20-22 years (43%) and in age groups 22-25 years were 32.8%, and students in age group ≥ 26 years count only 24.2% of the total (table1). The total OCC rate in our students was 17.7%. OCC was 26.7% among male students, higher than that for female students in which it was 9% (Table 2). In addition, there was a highly significant association of OCC with male students (OR= 3.7, $p=0.0001$) (table 3). When we study the relation of student age and risk of OCC, a higher rate and risk of colonization found in age group 23-25 years with prevalence rate equal to 23.8%, and risk (OR) equal to 1.65 times comparing to other age groups (table 2). When we considered predisposing factors of OCC. There was a highly significant association ($p=0.0004$) of denture wearing with OCC in which this risk equal to 6.2, and ranged from 1.8-22.2. Also proportional prevalence of 41% oral *C.albicans* was higher in students who had previously received antibiotics, and there was a highly significant association ($p<0.0001$) of history of recent using antibiotics with colonization of *C.albicans* in which this risk equal to 2.99, and ranged from 1.8 to 4.9. An inverse correlation between salivary flow rate and OCC is reflected in present study, in which significant relation was found between reduced saliva flow rate and OCC. Proportionally, 58.3% OCC was found with reduced saliva rate (< 1 ml/min) with highly significant OR equal to 14.6 times ($p<0.0001$). There was a highly significant association ($p<0.0001$) of smoking with OCC in which this risk equal to 14.6, and ranged from 6.5 to 32.9 (table 4). However, there was no effect for mouth hygiene in occurring of colonization of *C.albicans* among our students (table 4). In this study 29.1% of tested healthy students had oral colonization with Non-*Candida albicans* *Candida* colonization (ONCACC). In our study *Candida tropicalis* accounted for 10.2%, *Candida glabrata* for 11.7%, and *Candida parapsilosis* for 2.6% (table 2).

Discussion The total OCC rate in our students was 17.7%. This candidal carriage state is not considered a disease, but when *Candida* species become pathogenic and invade host tissues, oral candidiasis can occur. This change usually constitutes an opportunistic infection of because of local (i.e., mucosal, introducing oral devices as denture, dental bridge etc.), or systemic factors altering host immunity.[16] Our rate is slightly higher than these findings by Scully in UK in adults (11.5%), and by Tarcin in USA

(13%).[17,18] Also colonized of *C.albicans* was about 26.7% among male students, higher than that for female students in which it was 9% (Table 2). In addition, there was a highly significant association of *C. albicans* colonization with male students (OR= 3.7, $p=0.0001$) (table 3). Our result is different from that reported by Greenberg *et al.* [3] with high rate (40.9%) in adult females and low rate (12.2%) in adult males.

When we study the relation of student age and risk of mouth colonization of *C. albicans*, a higher rate and risk of colonization was found in age group 23-25 years with prevalence rate is equal to 23.8%, and risk (OR) equal 1.65 times comparing with other age groups. This result suggested that students in older age are an important risk factor of mouth colonization of *C.albicans*. Our result is different from that reported from Philadelphia by Bouquot *et al.*, [19] in which no different in the rate of mouth colonization occurred with age, but similar to that reported from UK by Smaancyake [20] in which the highest rate occurred in older adult age groups.

The second aim of the study was to determine other predisposing factors of OCC. There was a highly significant association ($p=0.0004$) of denture wearing with OCC in which this risk equal to 6.2, and ranged from 1.8-22.2 (table 4). This result can be explained by the fact that denture wearing, and poor denture hygiene, particularly wearing the denture continually rather than removing them during sleep [16], is another risk factor, both for candidal carriage and for oral candidiasis. Also dentures provide a relative acidic, moist and anaerobic environment because the mucosa coated by the denture is sheltered from oxygen and saliva.[17] Other cause for colonization of *C.albicans* in denture wearing persons is that loose, poorly fitting dentures may also cause minor trauma to the mucosa [20], which is thought to enhance the permeability of the mucosa and increase the ability of *C. albicans* to invade the tissues [17, 21]. These situations all favor the growth of *C. albicans*. Sometimes dentures become much worn, or they have been constructed to allow insufficient lower facial height (occlusal vertical dimension), directed to over-closure of the mouth (an appearance sometimes described as "collapse of the jaws"). This causes pronouncement of the skin folds at the corners of the mouth, in consequence creating an intertriginous areas where another form of candidiasis, angular cheilitis, can develop. *Candida* species are capable of adhering to the surface of dentures, most of which are made from polymethylacrylate. They exploit micro-fissures and cracks in the surface of dentures to aid their maintenance. Intra-oral prostheses may therefore become covered in a biofilm, [22] and act as reservoirs of infection [23], continually re-infecting the mucosa. For this reason, disinfecting the denture is a vital part of treatment of oral candidiasis in persons who wear dentures, as well as correcting other factors like not enough lower facial height and fit of the dentures.[17]

In fact broad-spectrum antibiotics used in the treatment of a wide range of disease conditions have also been recognized as a predisposing factor of OCC, possibly because of changes in oral environment and/or in the immune response reducing neutrophils candidacidal activity.[24] In this study, proportional prevalence of 41% oral *C.albicans* was higher in students who had previously received antibiotics. Also there was a highly significant association ($p<0.0001$) of history of recent using antibiotics with colonization of *C.albicans* in which this risk equal to 2.99, and ranged from 1.8 to 4.9 (table 4). This result can be explained by the fact that broad spectrum antibiotics lead to imbalance of the oral micro-organisms.[3, 25]

An inverse correlation between salivary flow rate and OCC has been reported [26] as is reflected in present study. In this study, significant relation was found between reduced saliva flow rate and OCC. Proportionally, 58.3% OCC was found with reduced saliva rate (< 1 ml/min) with highly significant OR equal to 14.6 times ($p < 0.0001$) (table 4). This association can be explained by that both the quantity and quality of saliva are important oral defenses against *Candida*. [27] Decreased salivary flow rate or a change in the composition of saliva [28] collectively termed salivary hypo function or hypo salivation is an important predisposing factor. Also *xerostomia* is frequently listed as a cause of candidiasis [16] but *xerostomia* can be subjective or objective, i.e., a symptom present with or without actual changes in the saliva consistency or flow rate.[16, 28]

There was a highly significant association ($p < 0.0001$) of smoking with OCC in which this risk equal to 14.6, and ranged from 6.5 to 32.9 (table 4). Our result is similar to that reported by Tarcin [17] in which a high significant risk of colonization was associated with smoking habit. This result can be explained by the fact that smoking, especially heavy smoking, is an important predisposing factor but the reasons for this relationship are unknown. One hypothesis is that cigarette smoke contains nutritional factors for *C. albicans*, or that local epithelial alterations occur that help colonization of *Candida* species [17] and smoking lead to kill immune cells and damage mucus membrane of the mouth.[24]

There was no effect for mouth hygiene in occurring of colonization of *C.albicans* among our students. This result is different from that reported by Rautema *et al.* [26] in which a high significant risk of mouth colonization was associated with bad mouth hygiene.

In this study 29.1% of tested healthy students had oral colonization with other *Candida* species than *C. albicans* (ONCACC) (table 2). In our study *Candida tropicalis* accounted for 10.2%, *Candida glabrata* for 11.7%, and *Candida parapsilosis* for 2.6% (table 2). Our result is similar to that reported elsewhere in which *Candida tropicalis* was the most common non-albicans species, followed by *Candida glabrata* while others ad *Candida parapsilosis* were rare isolated in healthy oral colonization or as cause of illnesses in patients.[9] As it is known *Candida* species may be capable of metabolizing ethanol to carcinogenic acetaldehyde and can thus progress oral and upper gastrointestinal tract cancer so 29.1% of our studied individuals under risk of oral and upper gastrointestinal tract cancer. Consequently, more focus should be placed on diagnosis and treatment of oral *Candida* infections, also on other *Candida* species than *C. albicans* as it has been recommended.[9,10,11,29, 30]

Conclusion

In the present study, the higher oral *Candida* carriage rate in healthy young adults buttresses the importance of oral *Candida* carriage for identification of individuals with the propensity for progression to clinical cases. Data from our study suggested that OCC was significantly associated with gender (male), smoking, denture wearing, dental bridge, orthodontics, the reduced saliva flow rate, previous antibiotics users, and Qat chewers. Our results are important for the development of strategies to eliminate these indicators of risk and significantly reduce OCC and oral *Candida* infections in young healthy adults and in general in Yemen community. The data also suggests that the prevalence rate of OCC was relatively high and it was affected by hygiene behaviors and certain

sociodemographic characteristics, which indicate the need for comprehensive, scheduled programs of healthcare educations.

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Table1: The distribution of tested students according to their sex and age groups

Age groups	Male n=131		Female n=134		Total n=265	
	NO.	%	No.	%	No	%
20-22 years	49	37.4	65	48.5	114	43
23 -25 years	40	30.5	47	35.1	87	32
≥ 26 years	42	32.1	22	6.4	64	24.2
Total	131	49.4	134	50.6	265	100
Mean age	23.4 years		22.1 years			
S. D	2.3 years		2.1 years			
Mode	22 years		21 years			
Median	21 years		21 years			
Max	27 years		26 years			
Min	20 years		20 years			

Table 2: The yeast distribution in different sexes of the study population

Organisms	Male n=131		Female n=134		Total n=265	
	No	%	No	%	No	%
<i>C.albicans</i>	35	26.7	12	9	47	17.7
<i>C.tropicalis</i>	16	12.2	11	8.2	27	10.2
<i>C.glabrata</i>	18	13.7	13	9.7	31	11.7
<i>C.parapsilosis</i>	4	3.1	3	2.2	7	2.6
<i>C.albicans C.tropicalis</i>	3	2.3	3	2.2	6	2.3
<i>C.albicans+ C.glabrata</i>	2	1.5	4	3	6	2.3
Non- <i>Candida albicans Candida</i>	43	32.8	34	25.4	77	29.1

Table 3: The prevalence and associated odds ratio of *Candida albicans* mouth colonization among different sexes and age groups

<u>Age groups</u>	Positive <i>C.albicans</i> (n=47)		OR	CI	χ^2	Pv
	No.	%				
Male n=131	35	26.7	3.7	1.74-8	14.3	0.0001
Female n=134	12	9	0.27	0.12-0.57	14.3	0.0001
Age groups						
20 -22 years n=114	15	13.2	0.56	0.3-1.15	2.9	0.09
23-25 years n=87	20	23	1.67	0.83-33.4	2.5	0.11
≥ 26 years n=65	12	18.5	1.1	0.48-2.3	0.03	0.86
Total n=265	47	17.7				

OR odds ratio > 1 (risk)

CI Confidence intervals 1 to more than 1

 χ^2 Chi-square > 3.9 (significant)

PV Probability value < 0.05 (significant)

Table 4: The risk factors of contracting *Candida albicans* mouth colonization among different student groups

Factors	Positive <i>C.albicans</i> (n=47)		OR	CI	χ^2	Pv
	No.	%				
Mouth hygiene						
Good n=115	16	13.9	0.67	0.4-1.2	2.03	0.15
Bad n=150	31	20.7	1.6	0.8-3.3	2.03	0.15
Antibiotic use n=39	16	41	2.99	1.8-4.9	17	<0.0001
Smoking n=48	28	58.3	14.6	6.5-32.9	66.2	<0.0001
Denture n=13	7	53.8	6.2	1.8-22.2	12.2	0.0004
Dental bridge n=19	8	42.1	5.4	1.7-16.99	12.2	0.0004
Orthodontics n=28	9	32.1	2.5	0.95-6.3	4.5	0.03
Qat chewing n=101	33	32.7	5.2	2.5-10.9	24.9	<0.0001
Saliva flow rate						
< 1ml/min n=63	31	49.2	11.3	5.2-24.5	56	<0.0001
> 2ml/min n=202	16	7.9	0.09	0.04-0.19	56	<0.0001
Halitosis (bad breath) n=67	22	32.8	3.4	1.7-6.9	14	0.0001

OR odds ratio > 1 (risk) CI Confidence intervals 1 to more than 1

 χ^2 Chi-square > 3.9 (significant)

PV Probability value < 0.05 (significant)